

C1 example, Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the AMERICAN TYPE CULTURE COLLECTION, 10801 University Boulevard, Manassas, VA. Suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978), SV-40, cytomegalovirus (U.S. Patent No. 4,956,288), and the adenovirus major late promoter. Expression vectors for use in mammalian cells include pZP-1 and pZP-9, which have been deposited with the AMERICAN TYPE CULTURE COLLECTION, 10801 University Boulevard, Manassas, VA under accession numbers 98669 and 98668, respectively, and derivatives thereof.

Please replace the paragraph at page 34, line 30 through page 35, line 9 with the following amended paragraph:

C2 A panel of cDNAs from human tissues was screened by PCR for zku6 expression. The panel included 77 cDNA samples from various normal and cancerous human tissues and cell lines as shown in Table 5. The panel was set up in a 96-well format that included a human genomic DNA (CLONTECH Laboratories, Inc., Palo Alto, CA) positive control sample. Each well contained approximately 0.2-100 pg/ $\mu$ l of cDNA. The PCR reaction mixtures contained oligonucleotide primers ZC28,995 (SEQ ID NO:8) and ZC28,996 (SEQ ID NO:9), *Taq* DNA polymerase (EXTAQ; TAKARA Shuzo Co. Ltd., Biomedicals Group, Japan), and a density increasing agent and tracking dye (REDILOAD, RESEARCH GENETICS, Inc., Huntsville, AL). The reaction mixtures were incubated at 94°C for 2 minutes; followed by 35 cycles of 94°C for 30 seconds, 61.4°C for 30 seconds, and 72°C for 30 seconds; followed by a 5-minute incubation at 72°C. About 10  $\mu$ l of each of the PCR reaction products was electrophoresed on a 4% agarose gel. The predicted DNA fragment size of ~110 bp was observed in brain, prostate, spinal cord, thyroid, fetal brain, placenta, salivary gland, testis, bone marrow, and stomach tumor, and possibly in islet, kidney, and HaCat cells.

Please replace the paragraph at page 35, lines 10-14 with the following amended paragraph:

C3 The DNA fragments for brain, prostate, fetal brain, and genomic DNA were excised and purified using a commercially available gel extraction kit (obtained from QIAGEN, Valencia, CA) according to the manufacturer's instructions. Fragments from fetal brain and genomic DNA were confirmed to be human zkun6 DNA by sequencing.

Please replace the paragraph at page 37, lines 3-30 with the following amended paragraph:

C4 The second-round positive pool was plated and transferred to nylon membrane filters (HYBOND-N; Amersham Pharmacia Biotech, Piscataway, NJ). Four filters at approximately 1000 colonies each were prepared. The filters were marked with a hot needle for orientation, then denatured for 6 minutes in 0.5 M NaOH and 1.5 M Tris-HCl pH 7.2. The filters were then neutralized in 1.5 M NaCl and 0.5 M Tris-HCl pH 7.2 for 6 minutes. The DNA was affixed to the filters using a UV crosslinker (STRATALINKER; STRATAGENE, La Jolla, CA) at 1200 joules. The filters were prewashed at 65°C in prewash buffer (0.25 x SSC, 0.25% SDS, 1mM EDTA). The solution was changed a total of three times over a 45-minute period to remove cell debris. Filters were prehybridized overnight at 65°C in 25 ml of a commercially available hybridization solution (EXPRESSHYB; CLONTECH Laboratories, Inc., Palo Alto, CA.). A probe was generated by PCR using oligonucleotide primers ZC29,898 (SEQ ID NO:10) and ZC29,899 (SEQ ID NO:11), a positive clone from the fetal brain library as template, an annealing temperature of 76.0°C, and 35 cycles. The resulting PCR fragment was gel purified using a commercially available kit (QIAQUICK gel extraction kit; QIAGEN). The probe was radioactively labeled with <sup>32</sup>P using a commercially available kit (REDIPRIME II random-prime labeling system; Amersham Pharmacia Biotech) according to the manufacturer's specifications. The probe was purified using a push column (NUCTRAP; STRATAGENE Cloning Systems, La Jolla, CA). Hybridization took place overnight at 65°C in a commercially available hybridization solution (EXPRESSHYB; CLONTECH Laboratories, Inc.). Filters were rinsed four times at 65°C in pre-wash buffer, then exposed to film for 3 days at -80°C. There were 6 positives on the filters. Six clones were picked from the positive areas and streaked out. Ninety-five individual colonies from these six positives were screened by PCR using oligonucleotide primers ZC29,898 (SEQ ID NO:10) and ZC29,899 (SEQ ID NO:11) and an annealing temperature of 61.0°C. Two positives were obtained. One clone (designated clone #1) was sequenced and found to include the 3' end and a sequence corresponding to the gap between the original ESTs.

Please replace the paragraph at page 37, line 31 through page 38, line 6 with the following amended paragraph:

C5  
To construct a full-length zkun6 cDNA, DNA was prepared from clone #1 and EST2906640 by the mini-prep method using a commercially available kit (obtained from QIAGEN). A 1015-bp 5'-end fragment was generated by digesting EST2906640 with EcoRI and AatII. A 1085-bp 3'-end fragment was generated by digesting clone #1 with AatII and XbaI. The two fragments were ligated to plasmid pZP-9, which had been digested with EcoRI and XbaI. The ligation mixture was transformed into *E. coli* strain DH10B™ (obtained from LIFE TECHNOLOGIES, Inc., Gaithersburg, MD) by electroporation. Ten clones were picked and checked by PCR using oligonucleotide primers ZC28,995 (SEQ ID NO:8) and ZC28,996 (SEQ ID NO:9) with an annealing temperature of 61.4°C. All clones were positive for the expected ~110-bp band. One clone was sequenced and confirmed to encode human zkun6.

Please replace the paragraph at page 38, lines 12-17 with the following amended paragraph:

C6  
11-day and 15-day mouse embryo cDNAs were screened for zkun6 by PCR using oligonucleotide primers ZC37,161 (SEQ ID NO:12) and ZC37,160 (SEQ ID NO:13) and *Taq* DNA polymerase (EXTAQ DNA polymerase; TAKARA Biomedicals) plus antibody. The reactions were run at an annealing temperature of 62.8°C with an extension time of 30 seconds for a total of 35 cycles. Products of both reactions were positive.

Please replace the paragraph at page 38, lines 18-27 with the following amended paragraph:

C7  
The mouse 15-day embryo library was screened for a full-length clone. This library was an arrayed library representing  $9.6 \times 10^5$  clones made in the vector pCMVSPORT2 (LIFE TECHNOLOGIES, Gaithersburg, MD). A working plate containing 80 pools of 12,000 colonies each was screened by PCR using oligonucleotide primers ZC37,161 (SEQ ID NO:12) and ZC37,160 (SEQ ID NO:13) with an annealing temperature of 62.8°C for 35 cycles. There were 3 positives. Pools corresponding to positive pools from the working plate were screened by PCR using the same reaction conditions. Four positives was obtained. Corresponding pools from the original source plates were then screened by PCR using the same reaction conditions. Reaction products were sequence and determined to represent mouse zkun6 DNA.